

Figure 1A

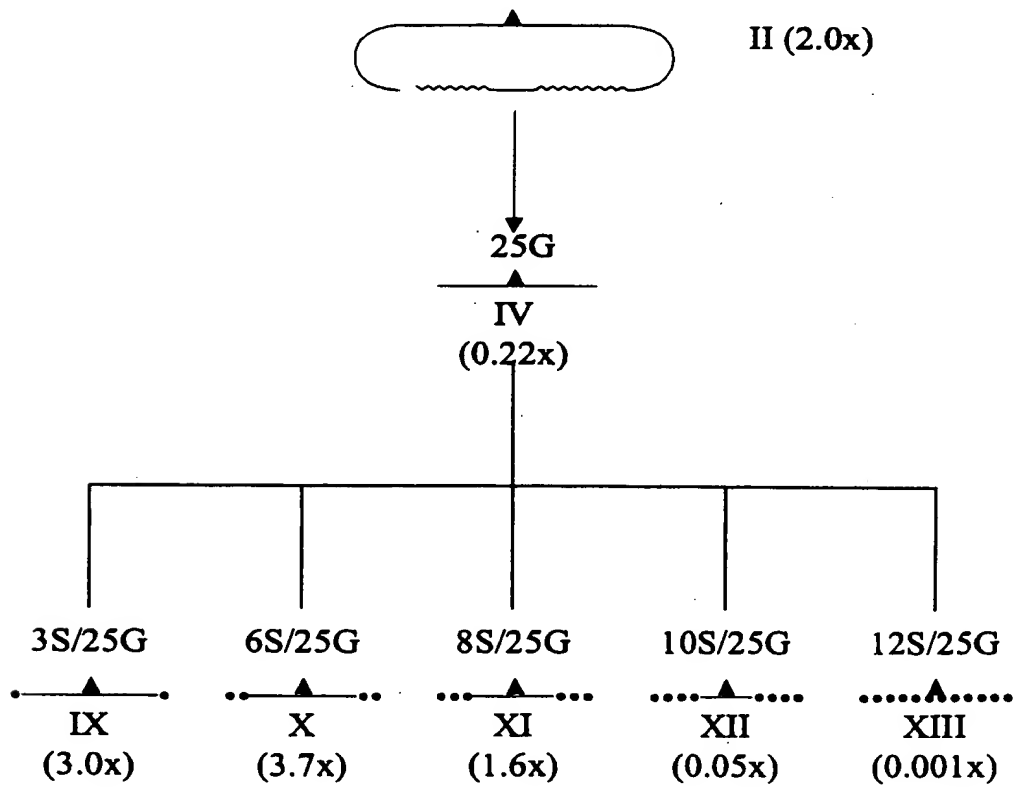
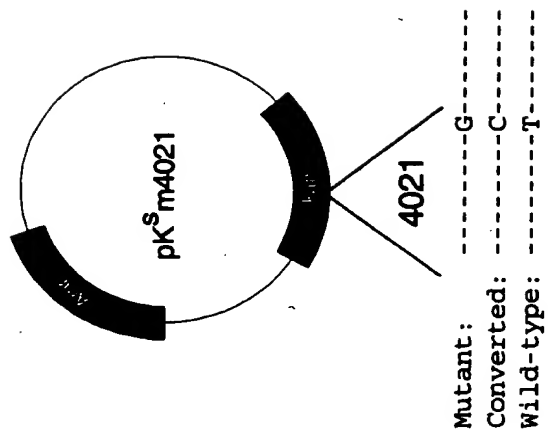
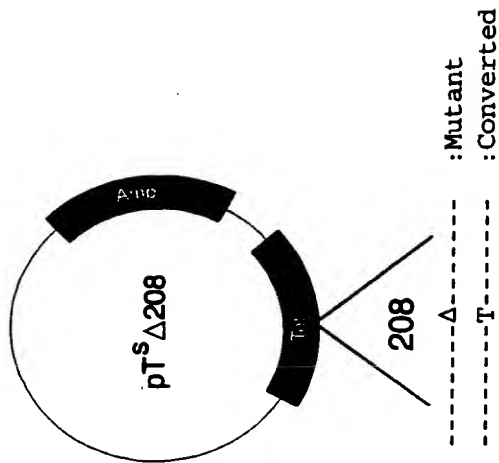
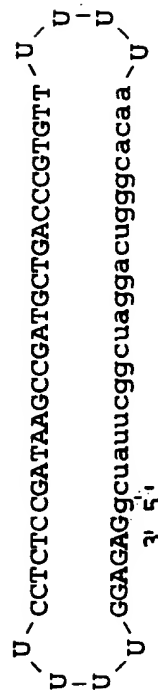


Figure 1B

# Plasmids, DNA targets and chimeric oligonucleotides



KanGG



Tet $\Delta 208T$

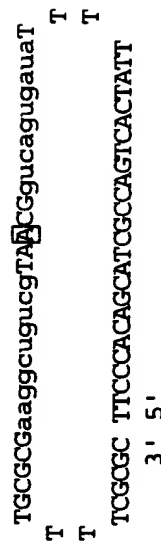
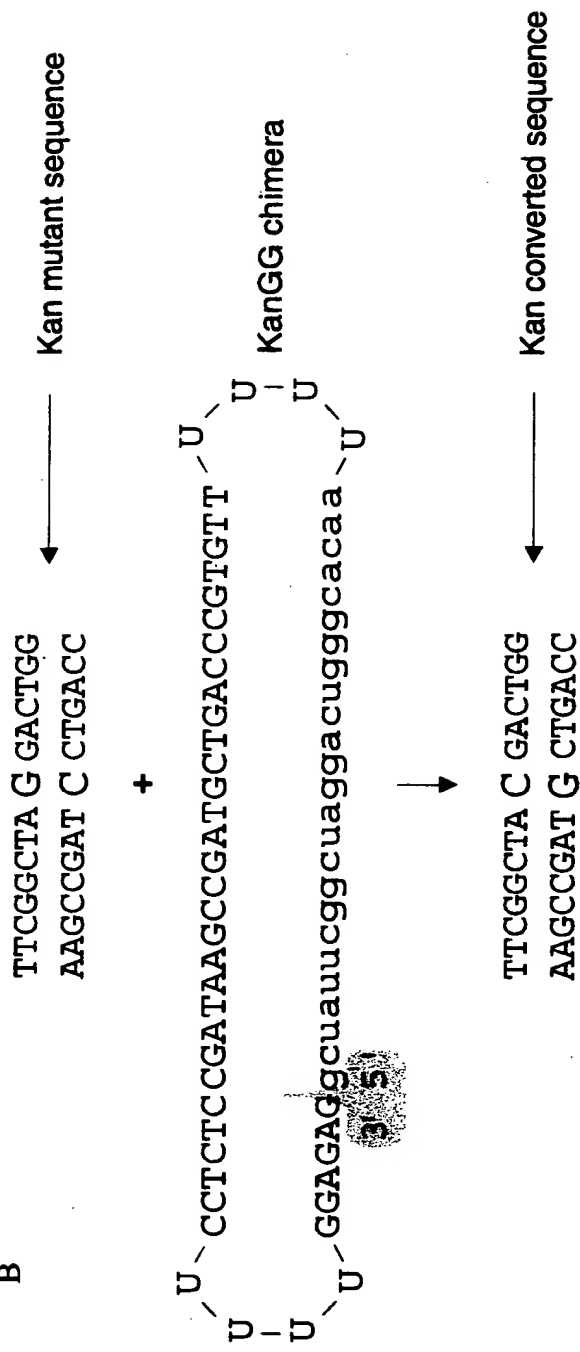


Figure 1C

bioRxiv preprint doi: <https://doi.org/10.1101/000000>; this version posted January 1, 2015. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

B



# DNA SEQUENCE ANALYSES

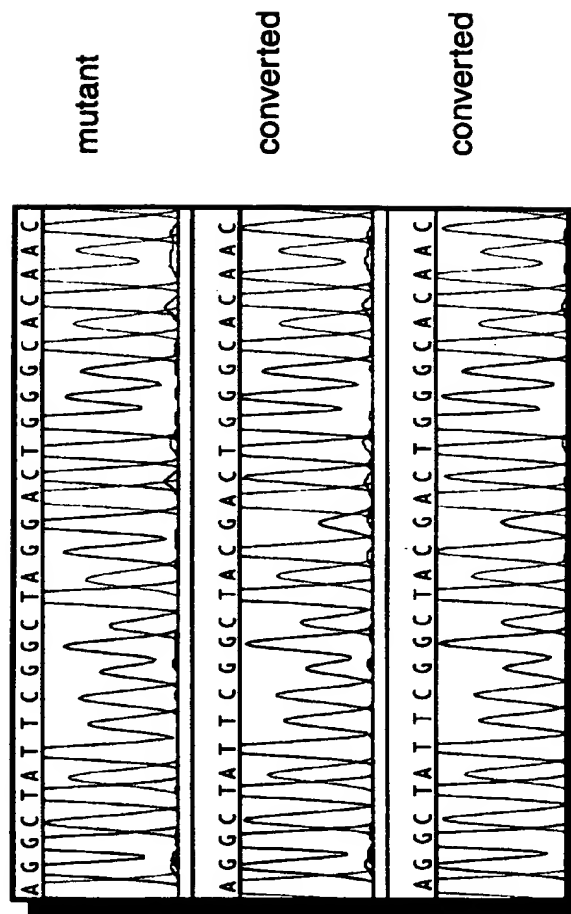


Figure 1D

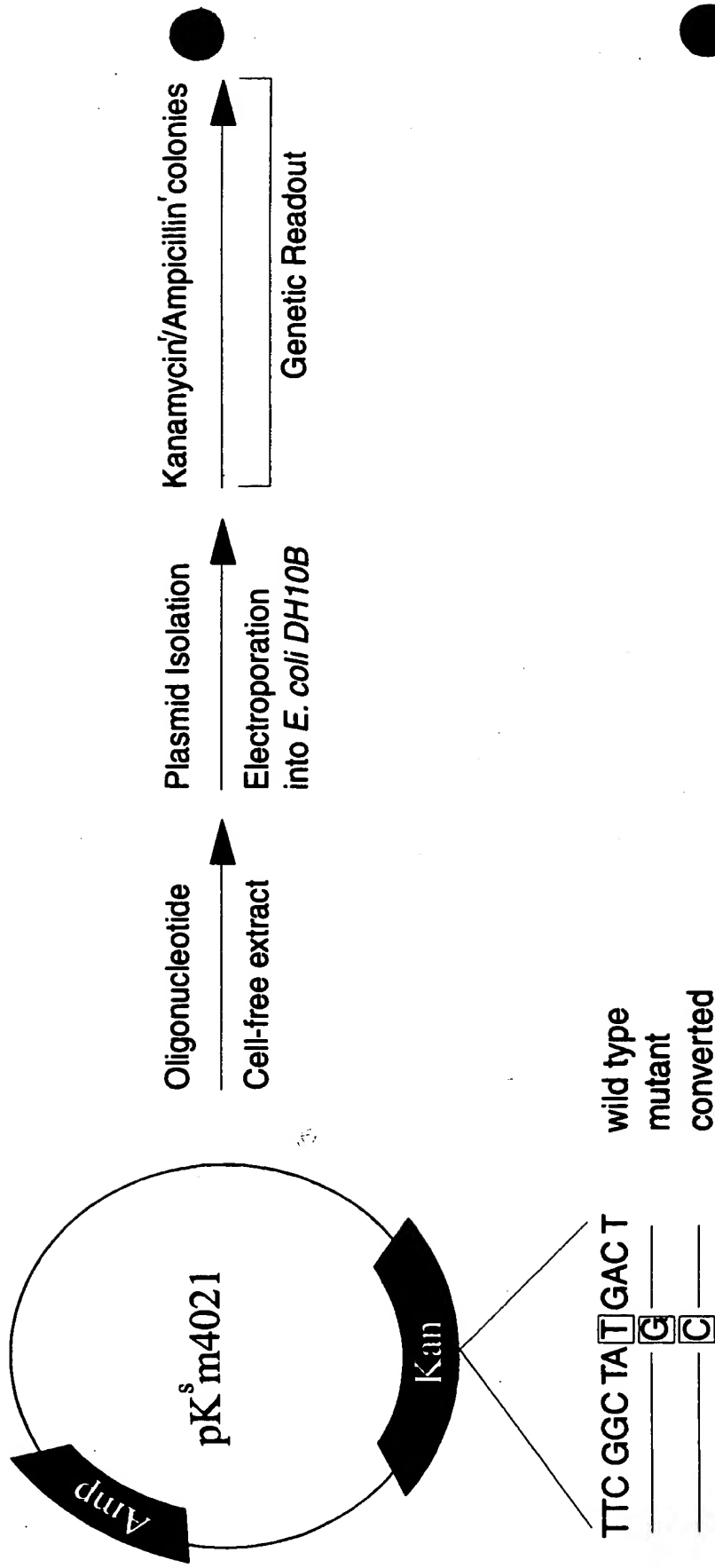
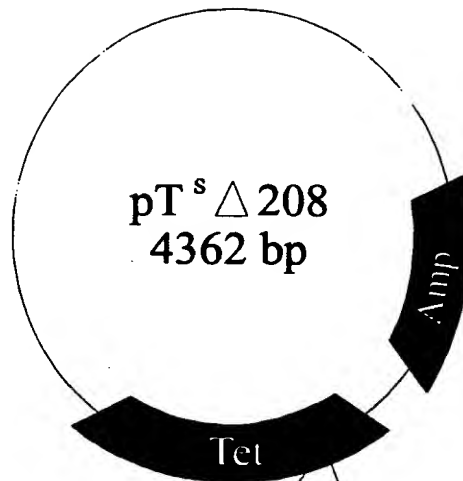


Figure 2

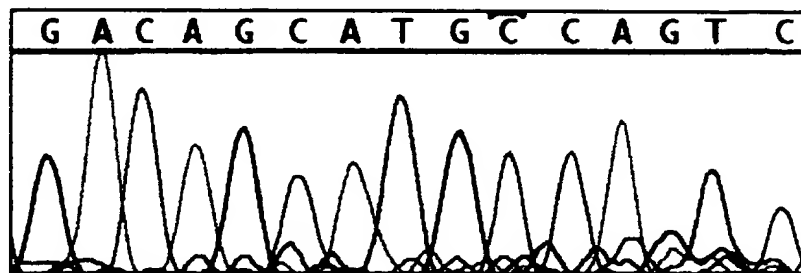


#208

Wild type: GAC AGC AT C GCCAGT  
 Mutant: GAC AGC AT - GCCAGT  
 Converted: GAC AGC AT T GCCAGT

### Sequence analysis of Tet<sup>r</sup> plasmid Δ208

Control



3S/25A

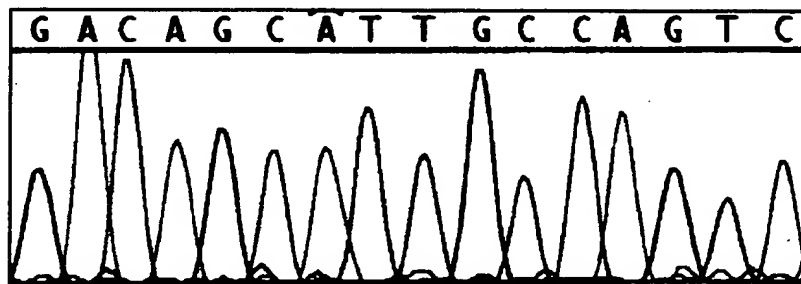
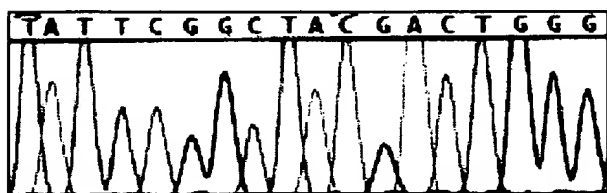


Figure 3

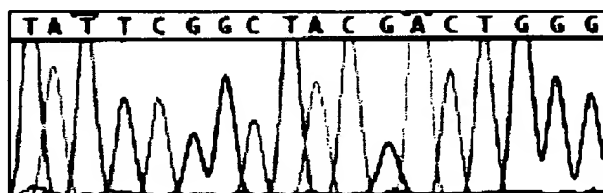
# DNA sequence analysis of Kan<sup>r</sup> plasmids

Target codon distribution					
oligomer	TAG	TAC	TAC/TAG	TGG	TCG
1) 3S/25G (20)	----	+	---	---	---
2) 6S/25G (20)	----	+	---	---	---
3) 8S/25G (20)	----	+	---	---	---
4) 10S/25G (18)	----	+	---	+(2)	+(2)
5) 25S/25G (4)	---	---	+(2)	+(2)	---

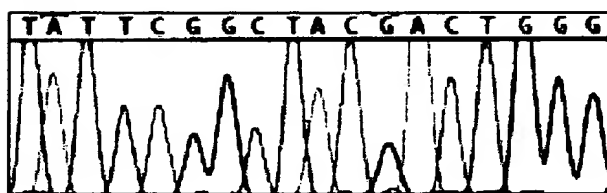
3S/25G



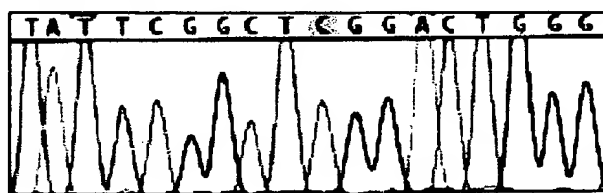
6S/25G



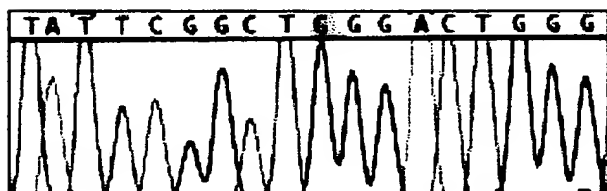
8S/25G



10S/25G



25S/25G



25S/25G

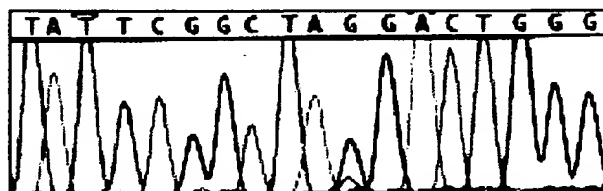


Figure 4

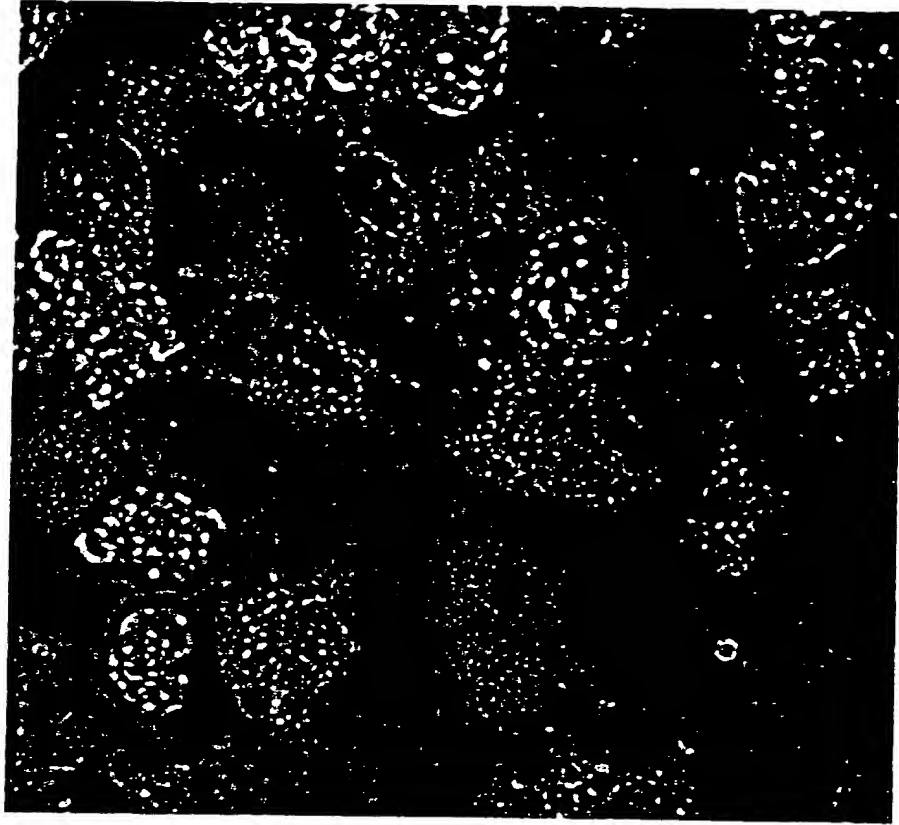


Figure 5

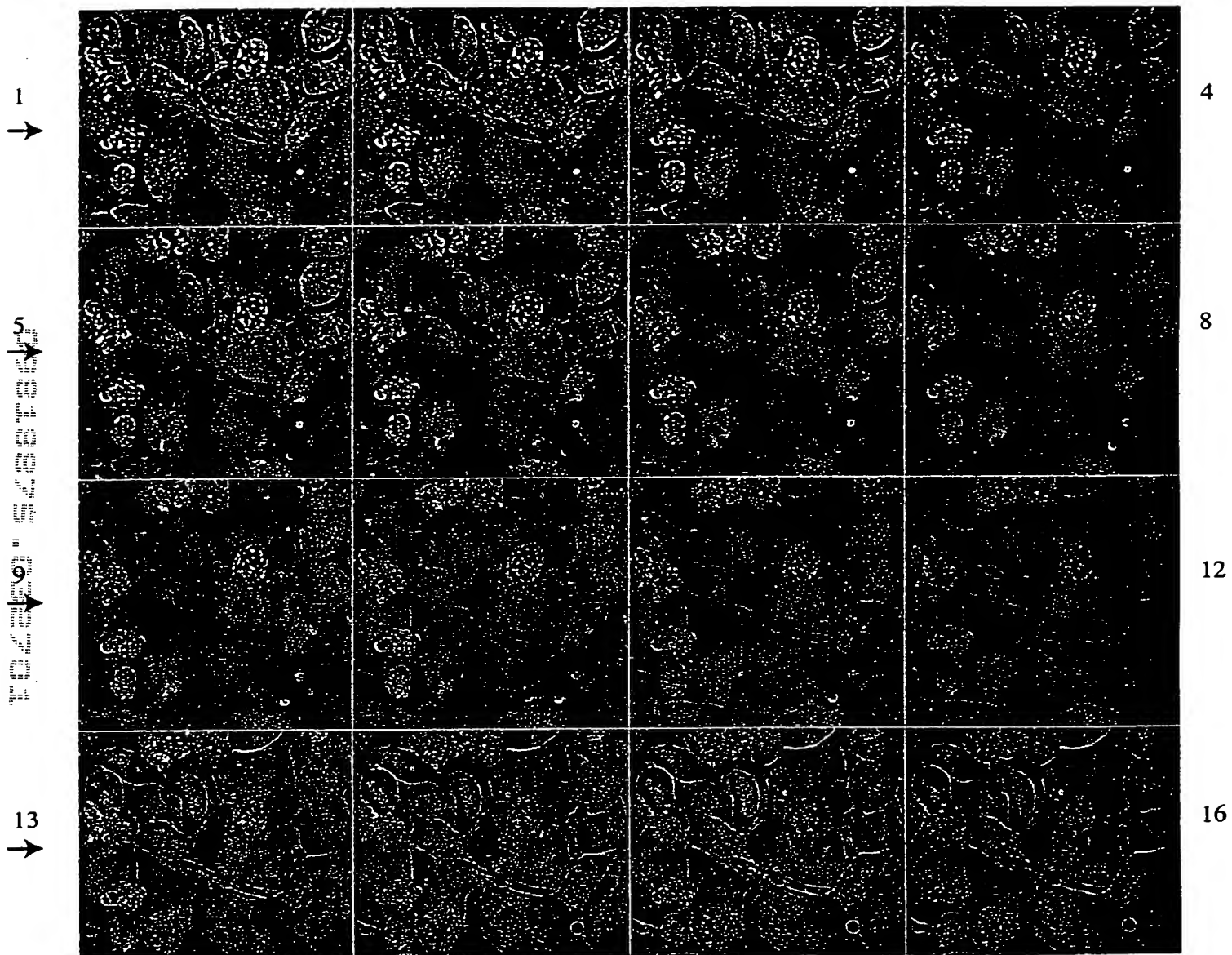


Figure 6



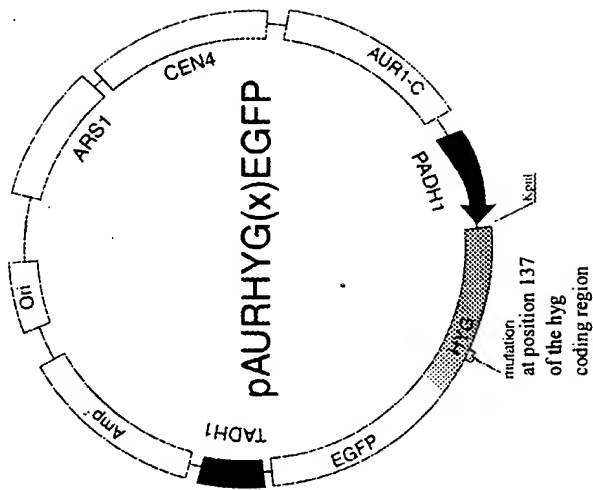


Figure 7A

Sequence of normal allele: GTGGATATGTCCT  
 Target/existing mutant: GTGGATAATGTCCT  
 Desired alteration: GTGGATACGTCCT

Sequence of normal allele: GTGGATATGTCCT  
 Target/existing mutant: GTGGATAGGTCCCT  
 Desired alteration: GTGGATACGTCCT

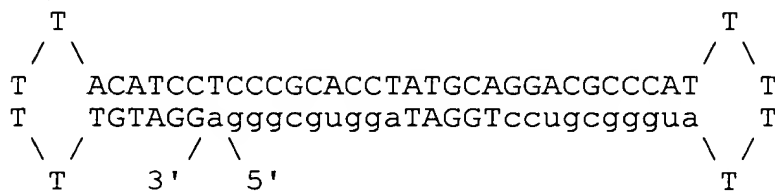
Figure 7B

HygE3T/25: 5'-AGG GCG TGG ATA CGT CCT GCG GGT A-3'

HygE3T/74: 5'-CTC GTG CTT TCA GCT TCG ATG TAG GAG GGC  
GTG GAT ACG TCC TGC GGG TAA ATA GCT GCG  
CCG ATG GTT TCT AC-3'

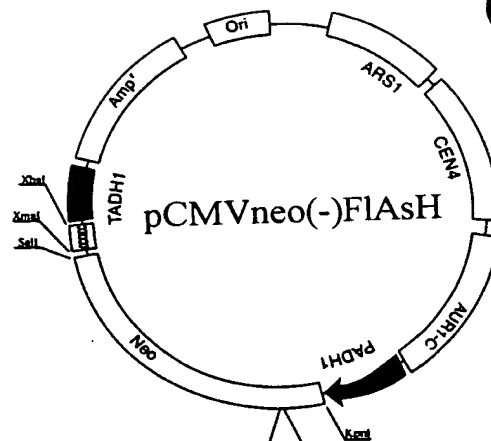
HygE3T/74α: 5'-GTA GAA ACC ATC GGC GCA GCT ATT TAC CCG  
CAG GAC GTA TCC ACG CCC TCC TAC ATC GAA  
GCT GAA AGC ACG AG-3'

HygGG/Rev:



Kan70T: 5'-CAT CAG AGC AGC CAA TTG TCT GTT GTG CCC AGT  
CGT AGC CGA ATA GCC TCT CCA CCC AAG CGG CCG GAG  
A-3'

Figure 8



TTCGGCTA G GACTGG  
AAGCCGAT C CTGACC

Neo/kan target  
mutant



TTCGGCTA C GACTGG  
AAGCCGAT G CTGACC

converted

### FUSION GENE FOR LIGAND BINDING

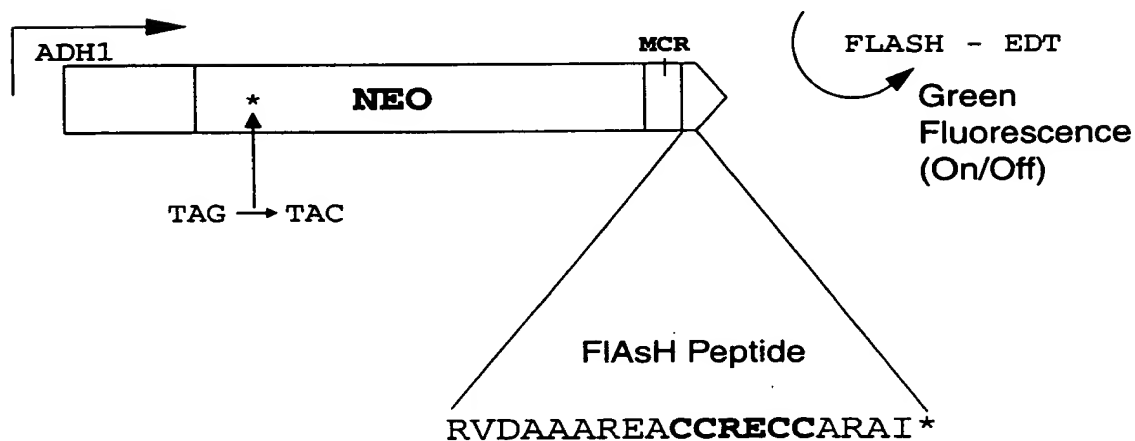


Figure 9

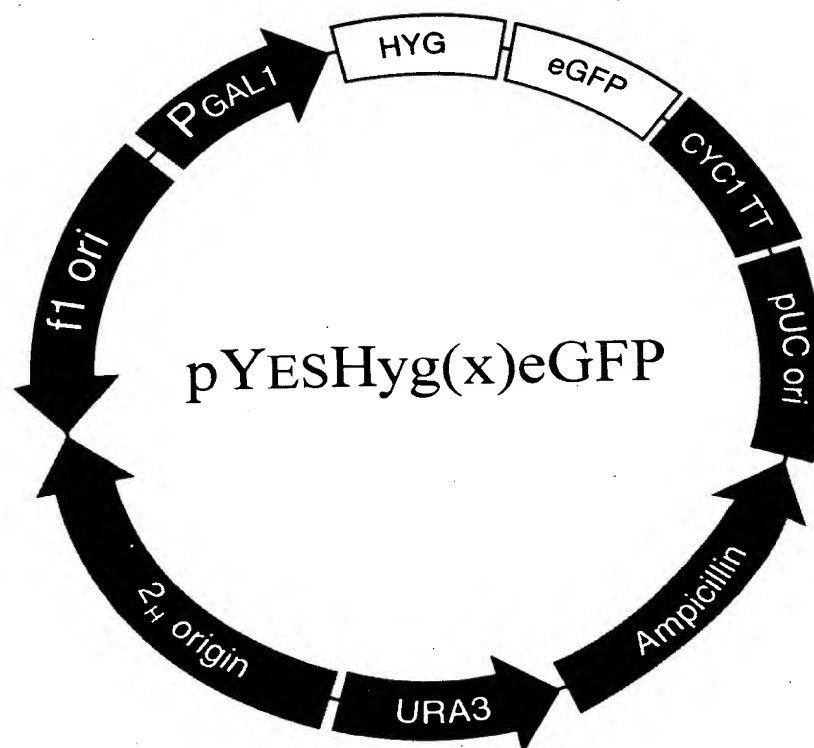


Figure 10